

# **Report on a Site Visit to Mexico – Muerte del Encino**

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## **Background**

Increased mortality of native oaks in montane forests of Mexico has been observed over about the last 15 years. The decline of the oaks is especially prevalent in the States of Colima, Jalisco, Aguascalientes, Guanajuato, and Nayarit. Symptoms vary, but typically include bleeding trunk cankers, crown dieback and decline, and mortality.

Although causal agents associated with the oak decline in all areas have not been investigated, bleeding cankers at the base of and along the main trunk of many of the declining oaks led pathologists to suspect *Phytophthora* as one possible cause. In some areas symptoms are similar to those reported for *Phytophthora*-infected oaks in Mexico (Tainter and others 2000), in Spain (Braiser and others 1993) and in California (Rizzo and others 2002). In one location (El Arrayanal) in the state of Colima, Tainter and others (2000) isolated *Phytophthora cinnamomi* from symptomatic trees and from surrounding soils, and demonstrated that inoculation of the native oak trees with *P. cinnamomi* resulted in canker formation.

In June 2003, Rob Mangold, Director of Forest Health Protection (FHP), received a request from José Cibrián Tovar, Director, Sanidad Forestal, Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT), for assistance in surveying parts of Mexican forests to determine the cause of the oak decline symptoms. Based on the presence of bleeding cankers on some symptomatic trees, *Phytophthora ramorum*, the cause of sudden oak death (SOD), was suspected as one possible causal agent.

The FHP International Activity Team compiled a USDA Forest Service team of pathologists to respond to the request from SEMARNAT. The team consisted of Dr. Jennifer Davidson, PSW, Davis; Dr. Joseph O'Brien, Northeastern Area, St. Paul; Steven Oak, Region 8, Asheville, and Dr. John Kliejunas, Pacific Southwest Region, Vallejo. The team was directed to work with Dr. Dionicio Alvarado-Rosales, Instituto de Fitosanida (IFIT), Colegio de Postgraduados (CP); and Engineer Oscar Trejo Ramirez, Centro Nacional de Referencia en Parasitología Forestal, SEMARNAT, to identify possible causal agents in areas suspected of having *Phytophthora ramorum*, in areas where that pathogen or other *Phytophthora* spp. might occur, and in areas of declining oaks.

In addition to the resources of the Forest Service team, Forest Health Protection provided funding for a grant awarded to Dr. Dionicio Alvarado-Rosales and Oscar Trejo Ramirez to survey, monitor and determine the impact of oak problems in Mexico. The plot

establishment part of that grant for monitoring progression of oak decline became part of the objectives of the team's site visit.

## **Objectives**

The objectives of the site visit were to:

1. Assist with establishment of long term monitoring plots in areas of declining native oaks in five Mexican States.
2. Provide support and training in field and laboratory techniques for isolation of *Phytophthora* species from oak trees and from soil.

## **Itinerary**

The site visit took place September 20 to October 3, 2003. The itinerary follows.

**Sunday September 21:** Forest Service team members Davidson, O'Brien, Oak and Kliejunas arrived in Guadalajara. Melissa Morris, graduate student at UC Davis, accompanied the team in their travels. The team met with Dr. Dionicio Alvarado-Rosales, Luz de Lourdes Saavedra-Romero (IFIT, CP), Silvia Garcia Diaz (Division de Ciencias Forestales de la Universidad Autónoma Chapingo), and Oscar Trejo Ramirez (SEMARNAT), to discuss and finalize the itinerary for the trip. Dionicio presented a brief history of the problem and discussed the objectives and logistics for the trip.

**Monday September 22:** The team (throughout the remainder of this report, "the team" refers to the United States group of Jenny, Joseph, Steve, John and Melissa; and the Mexican group of Dionicio, Oscar, Luz and Silvia) met at breakfast with Regional Director General Jaime Bocanegra, Comisión Nacional Forestal, SEMARNAT, to give a brief summary of objectives for the site visit. The team then traveled to the ejido of El Arrayanal in the state of Colima. Fernando Orozco, Dept. de Impact y Riesgo Ambiental, joined the group in Colima. El Arrayanal is the location of the work done by Tainter and others (2000) on *Phytophthora cinnamomi*. Joseph O'Brien had been to the area several times previously and was familiar with the history and progression of the oak decline in the area. The team met with the mayor and other citizens of El Arrayanal before going to the field. Several field sites in the area were examined as potential areas for plot establishment. One site had extensive oak defoliation, with suspected chafer beetle (Coleoptera: Scarabaeidae) feeding damage on the leaves. A soil sample was collected from that site and baited with pear and rhododendron. At a second site, soil and host tissues were sampled from one oak tree with canker symptoms similar to those caused by ink disease [Arrayanal gusher]. The team spent the night in cabanas provided by the government, in the mining town of Minatitlan.

**Tuesday September 23:** The team traveled to the Reserva de la Biofera Sierra Manantlán, in the state of Jalisco. After discussions on procedures for plot establishment and recording of tree condition, the first in a series of plots was established [Telcruz]. Bleeding cankers were common on the oaks in the Telcruz plot. A second plot [El

**Mango]** was established several kilometers away in the afternoon. The team then traveled to Manzanillo for the night.

The method of plot establishment and measurement used at Telcruz became the standard procedure at nine additional locations during the remainder of the site visit. The procedure follows:

A 0.1 ha permanent plot for long term monitoring of forest health was established in each of 10 areas (2 plots in each of the five states of Colima, Jalisco, Nayarit, Aguascalientes, and Guanajuato) (Fig. 1). The location of the plot within a stand varied, but if possible the plot was established on the margin of what appeared to an advancing front of declining trees. A plot center (a tree) was selected and each *Quercus* spp. within the 17.85 meter radius plot was painted with a number. Diameter at breast height, crown position, and degree of crown dieback were recorded using a system modified from that used for oak decline in the eastern United States (Starkey and others 1989, Oak and others 1990). The presence or absence of trunk cankers was noted. Soil samples were collected at five points within the plot. At each point (a point was an oak tree, usually one with trunk cankers), about a pint of soil from the fine root zone was collected at each of three locations around the tree, and placed in a labeled plastic bag. The five bags of soil from each plot were taken to Dionicio's lab in the Instituto de Fitosanidad, Colegio de Postgraduados, Monticello and baited for *Phytophthora* using leaf pieces of camellia and rhododendron (Ferguson and Jeffers 1999). Cankers from trees within and just outside the plot were sampled for *Phytophthora*. Pieces of tissue from canker margins were placed on PARP medium (Erwin and Ribeiro 1996).

**Wednesday September 24:** The team returned to the El Arrayanal area and established two plots. The first plot [**Arrayanal #1**] had oaks with bleeding cankers (Fig. 2), numerous wind thrown oaks with signs of *Armillaria*, insect borers in declining trees, and evidence of site disturbance including fire, tree removal, and grazing. The plot was established along what appeared to be an advancing edge of declining trees. After a visit to the site of the reported origin of the problem in the El Arrayanal area and examination of trees inoculated by Tainter, a second plot [**Arrayanal #2**] was established east of the town. The area was relatively rocky and dry. No cankers were observed on the oaks. The site was disturbed, with evidence of fire, tree removal and grazing.

On the return to Guadalajara, the team stopped at the SEMARNAT office in Colima for a closeout session with Salvador Díaz Saldaña, Secretario Técnico, (representing Delegado Federal Guillermo Romero Ibarra), SEMARNAT. We then continued on to Guadalajara for lodging.

**Thursday September 25:** The team left Guadalajara at 8:00 a.m. for a 3 hour drive to the Compostela area in the state of Nayarit. David Quiroz, SEMARNAT, accompanied the team. After examination of one stand with chafer defoliation, no cankers, and possible herbicide damage, a plot [**El Ranchito**] was established in a suitable stand. No stem cankers were observed. Anthracnose symptoms on twigs were abundant on many of the oaks and fruiting of *Hypoxylon* was present on some. Bleeding cankers were absent. A nearby area previously deemed suitable for plot establishment was not accessible with

our vehicles because of washed out roads. Local SEMARNAT staff members were shown what information to collect and how to sample soils. They accessed the site [Miravalles] the next day, established a plot, and collected soils that were mailed to Dionicio's lab. The team traveled to Tequila for dinner and returned to Guadalajara for the night.

**Friday September 26:** The team traveled to Aguascalientes, in the state of Aguascalientes. After an initial meeting with local SEMARNAT representatives, the team traveled to a site on a steep valley hillside and established a plot [Ranchito Piletas]. The plot was established at what appeared to be an advancing front of crown decline symptoms (Fig. 3). Many of the oaks had bleeding cankers, some of which showed evidence of oak borers.

A second plot was established on a flatter landscape above the valley [Mesa del Aguila]. The soils were shallow, and anaerobic. No active bleeding cankers were observed, though insect borers were present in some oaks. After establishment of the two plots, the team enjoyed a barbeque hosted by SEMARNAT and the local landowner. Following the barbeque, the team returned to Aguascalientes where it spent the night.

**Saturday, September 27:** We traveled to the Universidad Autónoma de Aguascalientes where we met with Dr. José de Jesús Luna Ruiz, Centro de Ciencias Agropecuarias; and Dr. Onesimo Moreno Rico, Depto. De Microbiología. We used the laboratory facilities to examine agar plates and to transfer suspect organisms to multiple media in order to obtain pure cultures. The afternoon was occupied by a seminar. About 35 faculty and students attended. Dionicio discussed the objectives of the team's visit and gave a background on the oak decline situation in Mexico. Presentations by members of the team followed. Presentations included a discussion of oak wilt by Joe O'Brien, a summary of the sudden oak death situation in California (in Spanish) by Jenny; and a discussion of oak decline in the eastern United States by Steve Oak. Following the seminar, the team drove to Leon where we spent the night.

**Sunday, September 28:** The team enjoyed a day off, visiting several sites (museo de las momias, Callejón del Beso, Alhondiga de Granaditas, and el Pípila) in the town of Guanajuato, a world heritage site and capitol of the state of Guanajuato.

**Monday September 29:** We traveled north from Leon to Sierra Fria in the state of Guanajuato and established the 1<sup>st</sup> plot [Pozo Redondo] of the day. The area was grassy, with evidence of heavy grazing, fire and tree removal. Insect borers and defoliation were present on some of the oaks. Fruiting of *Ganoderma* was common. A tree with evidence of *Ganoderma* and *Hypoxylon* was cut and examined. Another tree was cut off the plots and discs taken for isolations in the laboratory. An oak with bleeding cankers was cut down and samples taken.

We then drove to a 2<sup>nd</sup> site [Vergel de la Sierra]. This site was similar to the first, with evidence of grazing and past cutting disturbance. Insect borers were observed in some oaks. *Ganoderma* was less common than at the first plot, but was fruiting on cut stumps.

Sprout regeneration was noticeably absent. After traveling back to Leon, we met with Delegado Federal Roberto Acero Rueda for a brief discussion of the progress of the site visit to date.

**Tuesday September 30:** Most of the day was required to travel the 340 km from Leon to Texcoco, in Mexico State. Texcoco is approximately 35 km east of Mexico City. The U.S. group stayed at the La Mansion del Quijote.

**Wednesday October 1:** The team went to the Universidad Autónoma Chapingo, where we met with Dr. David Cibrián Tovar and Rodolfo Campos Bolaños, Division de Ciencias Forestales, and with Dr. Fernando Zavala, oak taxonomy expert. Dr. Zavala identified samples of *Quercus* spp. we had collected from various locations. Identification of the native Mexican *Quercus* is a challenge, with over 150 different species named and hybridization common.

We then traveled to Dionicio's lab at the Instituto de Fitosanidad, Colegio de Postgraduados, Monticello. The soils collected were set up for baiting with camellia and rhododendron leaves. We met Dr. Bertha Tlapal-Bolaños, Instituto de Fitosanidad, an expert on *Phytophthora* species, who will be participate in the grant with Dionicio. We returned to the University in Chapingo for a 5:00 p.m. seminar. Approximately 65 students and faculty attended the seminar. As at Aguascalientes, presentations included a discussion of oak wilt by Joe O'Brien, a summary of the sudden oak death situation in California (in Spanish) by Jenny; and a discussion of oak decline in the eastern United States by Steve Oak. Melissa Morris translated the presentations by Joe and Steve. Dionicio presented a summary of the oak decline problem in Mexico and of the team's objectives. We met Jose Cibrián Tovar, Director, Sanidad Forestal, SEMARNAT.

**Thursday October 2:** The team returned to the labs of Dr. David Cibrián Tovar, where David discussed the progress on the book "Forest Diseases of Mexico". The book, begun in 1997, is to be a companion book to the "Forest Insects of Mexico" book published in 1995. The book is near completion and expected to be ready for printing in several months. Chapters by 13 authors covering 157 pathogens are completed. All illustrations, by Leticia Arango Caballero, are finished, and translation of the text from Spanish to English has begun.

We returned to the Colegio to remove baits that had been in soil suspension for 24 hours. The baits were plated on PARP amended with hymexazol. The remainder of the baits were to be removed and plated after 72 hours by Dionicio and his students. The U.S. group met with Dr. Ignacio Cid del Prado Vera, Director, Instituto de Fitosanidad, Colegio de Postgraduados, for a closeout session. Dr. Vera completed his doctorate at the University of California in Davis.

After a brief visit to the Universidad Autónoma de Chapingo Chapel, to view the murals by Diego Rivera adorning the walls and ceiling, the agar plates and samples collected during the trip were mailed via Fed Ex to Jenny's lab in Davis. The team then traveled to Teotihuacan, a pre-Columbian city 30 miles outside Mexico City. The city was

established several hundred years B.C. At one time, an estimated 200,000 people lived in the city, but by the 8th century it was abandoned. The zone covers about 5 square miles and countless structures, chief among them the Pyramids of the Sun and Moon. The team climbed the Pyramid of the Sun, a huge construction made of stone and mud that measures 225 meters around its base and 63 meters of slope with stairs on the center of the pyramid all the way to the top.

The team traveled to a hotel adjacent to the Mexico City airport for the night.

**Friday October 3:** Members of the Forest Service group returned to the United States.

### **Results of Isolations and Conclusions**

*Phytophthora cinnamomi* (identified by DNA analysis at Dr. Davidson's laboratory in Davis) was recovered from bleeding cankers on three *Quercus peduncularis* trees at Telcruz and from the one *Quercus* sp. tree with symptoms of ink disease near El Arrayanal (Arrayanal gusher). Further, the same organism was recovered from the 24-hour baits of soils taken from those two locations, and the 24-hour baits of soils from Arrayanal #1 and Arrayanal #2. Recoveries of *P. cinnamomi* from these El Arrayanal locations confirm the findings of Tainter and others (2000). Results are summarized in Table 1. The significance of the unidentified Oomycete is not clear since it was only recovered from soil and not from oak tissue.

Several interesting results require further investigation. Although *P. cinnamomi* was recovered from soil at Arrayanal #2, no bleeding cankers were observed on oaks at that site. Also, several of the plots were established in areas where the oaks had bleeding cankers, but *P. cinnamomi* was not recovered from oak tissue or soil. These areas include Ranchito Piletas in the state of Aguascalientes and Pozo Redondo in the state of Guanajuato. Periodic re-examination of the long term monitoring plots will provide additional information on the causes of the observed oak decline in the five Mexican states.

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Fig. 1. Mexican states where long-term monitoring plots were established.



Fig. 2. Bleeding canker on oak, El Arrayanal. Fig. 3. Oak decline, Ranchito Piletas.



Table 1. *Phytophthora* isolations from oak trunks and soil at 12 sites in five Mexican states.

State	Plot	Site	Isolations from Quercus	Isolations from Soil
JALISCO	1	Telcruz (Manantlan Biosphere)	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>
	2	El Mango	0	0
COLIMA		El Arrayanal by "gusher" oak tree	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>
		El Arrayanal on leaf chafer site	0	Unidentified Oomycete
	3	Arrayanal #1	0	<i>P. cinnamomi</i>
	4	Arrayanal #2	0	<i>P. cinnamomi</i>
NAYARIT	5	El Ranchito	0	0
	6	Miravalles	0	0
AGUASCALIENTES	7	Mesa del Aguila	0	0
	8	Ranchito Piletas	0	0
GUANAJUATO	9	Pozo Redondo	0	0
	10	Vergel de la Sierra	0	0